The Perchlorate Discharge Test for Examining Thyroid Function in Rats

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A perchlorate discharge test was developed for rats to detect changes in the thyroidal iodide accumulation and organification mechanisms. Rats were pretreated with compounds that alter thyroid function by different mechanisms: SK&F 93479 (an H_2-antagonist that enhances pituitary thyroid stimulating hormone drive by increasing thyroid hormone clearance) and propylthiouracil (an inhibitor of iodide organification). Six hours following administration of ^{125}I, either potassium perchlorate (10 mg/kg × 2.5 min) or saline was given i.p. Perchlorate significantly reduced the thyroid:blood ^{125}I ratio in propylthiouracil-treated rats but had no effect in those pretreated with SK&F 93479, indicating an iodide organification block in the former. At the same time thyroidal radioiodide accumulation in SK&F 93479-treated rats (no perchlorate) was enhanced, whereas that in propylthiouracil-treated animals (no perchlorate) was depressed.

Key Words: Perchlorate test; Thyroid; Rat

INTRODUCTION

The efficiency of the thyroid iodide organification mechanism in man can be monitored by the perchlorate discharge test for conditions such as Hashimoto's disease (Gray et al., 1974; Hilditch et al., 1980) and for assessment of the effectiveness of carbimazole (Low et al., 1979). Perchlorate is a competitive inhibitor of thyroidal iodide transport (Halmi et al., 1956), and if free iodide is backed up within the thyroid cells following perchlorate administration, there is a diffusional discharge of iodide. For investigation of the possible antithyroid actions of drugs, such a test would be useful in rodents. In this study we have developed the test for rats to act as an indicator of both thyroid iodide accumulation and organification efficiency. Two drugs were investigated: 1) SK&F 93479, and H_2 antagonist that increases thyroxine clearance from the circulation and thus thyroid stimulating hormone (TSH) drive to the thyroid gland (indirect action on the thyroid gland (Brown et al., 1986a)]; and 2) propylthiouracil (PTU) an inhibitor of the thyroidal peroxidase enzyme (direct antithyroid action on the gland).

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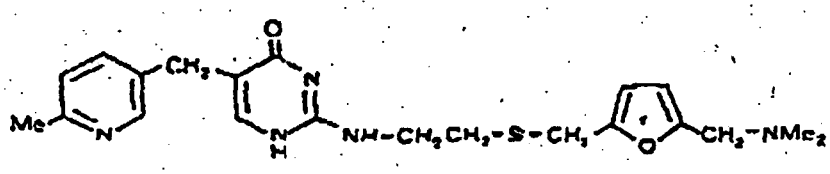
METHODS

Drug Pretreatment

The SK&F Wistar rats were treated with either distilled water (2 ml p.o.), PTU (50 mg/rat p.o.), or SK&F 93479 (1 g/kg p.o.) for 7 days. At 24 hr following the last dose, perchlorate discharge studies were performed. The structure of SK&F 93479 is as follows:



Perchlorate Discharge Test

At time = 0 min all rats were dosed with 1 μCi ^{125}I (100 μl, i.p., Amersham International plc). After 6 hr, each drug-treated group was divided into two subsets, one of which received saline (i.p.) and the other, potassium perchlorate ($KClO_4$). After 2.5 min (see below) rats were sacrificed; a 1-ml blood sample was taken from the posterior vena cava; and the thyroids were removed, weighed, and counted (see Figure 1 for flow diagram). The thyroid:blood ^{125}I (T:B) ratio was then calculated as counts per minute per gram of tissue per milliliter of blood.

Optimization of Test

Following administration of ^{125}I to rats for 0–9 hr, there was a progressive increase in thyroidal radioiodide accumulation with time (Table 1) with no maximum achieved within 9 hr. A 6-hr time point was chosen for most of the subsequent

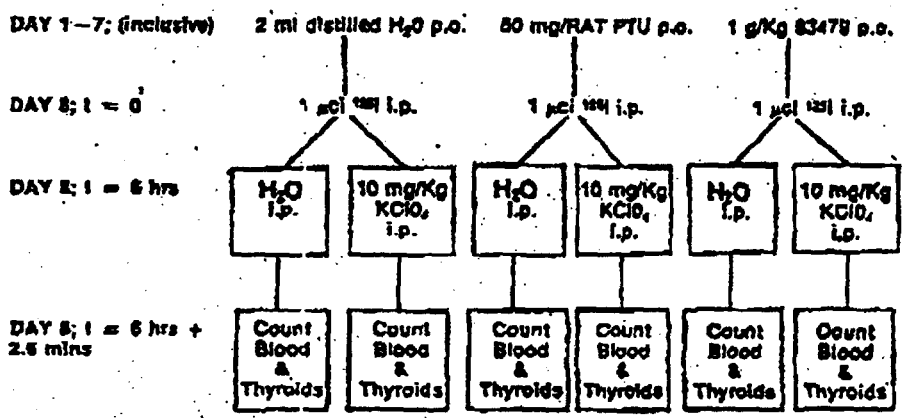


FIGURE 1. Rat perchlorate discharge test method.

TABLE 1 Time Course of ^{125}Iodide Accumulation by the Rat Thyroid Gland

^{125}I Accumulation Time (hr)	Mean Thyroid:Blood ^{125}I Ratio[a] ± SEM	n
1.0	4.19 ± 0.3	6
3.0	12.01 ± 1.0	6
6.0[b]	21.70 ± 2.0	6
9.0	48.34 ± 5.3	6

[a] Counts per minute per gram of tissue per milliliter of blood.
[b] Time chosen for future work.

TABLE 2 Effect of Perchlorate Dose Level on ^{125}I Uptake by the Rat Thyroid (3- or 6-Hr Accumulation)

Perchlorate Dose (mg/kg) Given at + 3 hr	4-hr ^{125}I Accumulation[a] Thyroid:Blood Ratio[b]	7-hr ^{125}I Accumulation[a] Thyroid:Blood Ratio[b]
0	16.82 ± 0.8	26.47 ± 3.1
5	9.36 ± 1.2	22.36 ± 1.8
10[c]	7.82 ± 1.0	18.79 ± 1.4
25	7.19 ± 0.8	16.32 ± 1.2
50	5.85 ± 0.7	16.07 ± 0.78

[a] $KClO_4$ additional dose time = +1 hr; n = 5–6 rats per group.
[b] Counts per minute per gram of thyroid per milliliter of blood.
[c] Dose chosen for future work.

TABLE 3 Effect of Perchlorate Time on Radioiodide Discharge by the Rat Thyroid Gland[a]

Treatment	Time Additional with $KClO_4$ (Min)	Control Thyroid:Blood ^{125}I Ratio (%)
Control (2 ml H_2O/day p.o. × 7 days	0	100
	1.0	109.2
	2.5[b]	96.2
	5.0	93.8
PTU (50 mg/rat p.o.) per day × 7 days	0	100
	1.0	110.3
	2.5[b]	43.5
	5.0	30.8

[a] $KClO_4$ dose = 10 mg/kg i.p.; ^{125}I accumulation time = 6 hr.
[b] Chosen time for future work.
[c] Propylthiouracil.

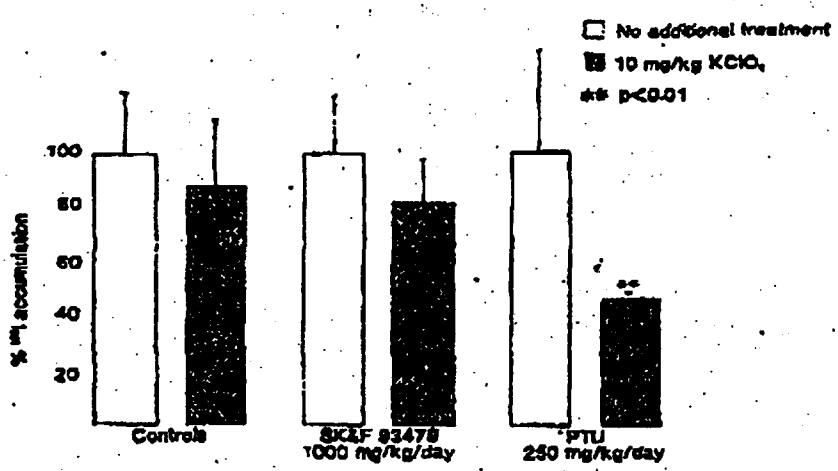


FIGURE 2. Effect of potassium perchlorate on ^{125}I discharge from the thyroid glands of rats pretreated with SKF 93479 (1000 mg/kg × 7 days) or PTU (50 mg/rat × 7 days). At 24 hr following the last dose, each treatment group was divided into two subsets (n = 8–10), and both were given ^{125}I (1 μCi). After 6-hr, one subset (open histogram) was given saline i.p., and the other (filled histogram) was given $KClO_4$ (10 mg/kg i.p.); 2.5 min later, rats were killed, thyroids were removed, weighed, and counted, and a sample of blood was also counted. Results are expressed as percentages of T:B ratio ± S.D. of rats given $KClO_4$ against subset not receiving $KClO_4$. Statistics were performed using Student's t-test (unpaired).

studies. Different doses of $KClO_4$ (5–50 mg/kg) were first tested for effectiveness in inhibiting iodide accumulation (3- and 6-hr accumulation) where all doses of perchlorate blocked ^{125}I uptake with 10 mg/kg appearing to be the dose producing the maximum effect (Table 2). Beyond the 10- to 25-mg/kg plateau, a further nonspecific decrease was seen (perchlorate exposure for 1 hr was used in these studies). Using 10 mg/kg $KClO_4$ the optimum time for thyroidal exposure to perchlorate was examined (Table 3). After 1 min no discharge of ^{125}I from either control or PTU-treated rats occurred, but following 2–5 min there was a large reduction (60%) in ^{125}I accumulation by thyroids from PTU-treated rats, where because of iodide organification block one would expect a perchlorate-induced discharge.

RESULTS

Control rats showed no significant reduction in thyroidal radioiodide accumulation (Figure 2) on administration of $KClO_4$. In contrast, PTU led to a large ^{125}I discharge when perchlorate was given (Figure 2). Comparing the absolute T:B ^{125}I ratio for the PTU subset not receiving $KClO_4$ (T:B = 237.5 ± 26.5 cpm/g/ml, n = 8) against control rats not receiving perchlorate (T:B = 2145.6 ± 175.6 cpm/g/ml; n = 8), there was also a marked reduction in ^{127}I accumulation. The perchlorate

discharge test showed no differences between rats pretreated with SK&F 93479 and controls (Figure 2), but again data from animals not receiving perchlorate indicated an enhancement of thyroidal ^{125}I accumulating ability due to SK&F 93479 treatment (T:B = 3428 ± 263.8 cpm/g/ml, $n = 8$; $p < 0.01$ significantly different from controls).

DISCUSSION

It has been shown here that the perchlorate discharge test is potentially useful for detecting simultaneous changes in the ability of the rat thyroid gland to accumulate and organify iodide. Optimal parameters for use of this test in rats have been defined (6-hr ^{125}I accumulation time; 10 mg/kg i.p. $KClO_4$ dose; 2.5-min $KClO_4$ exposure time). Following $KClO_4$ administration to control rats, there was no discharge of radioactivity from the thyroid cells, which suggested that after a 6-hr period virtually all the accumulated radioiodide had been organified into thyroid hormone. In contrast, PTU treatment, which inhibits the thyroidal peroxidase enzyme, caused a large perchlorate-induced discharge of free radioiodide from the follicular epithelial cells. Although this treatment increases TSH drive to the thyroid gland and thus cellular activity, the absolute ^{125}I accumulation was lower since accumulated iodide is not retained within the cells. The SK&F 93479 treatment increases peripheral thyroxine clearance in rats, thus enhancing pituitary TSH drive to the thyroid cells and concomitant radioiodide accumulation (Brown et al., 1986a). In this case, however, perchlorate administration did not cause a radioiodide discharge indicating normal iodide organification. This result is consistent with observations that SK&F 93479 treatment does not inhibit iodide organification in vitro by cultured porcine thyrocytes (Brown et al., 1986b). Thus, the potential of this test to reveal iodide organification defects in rats following drug treatment has been demonstrated, as has the ability to differentiate between agents having direct or indirect toxicological effects on the rat thyroid gland.

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